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PRINCIPAL INVESTIGATOR: Erin McCoy

CONTRACTING ORGANIZATION: University of Alabama at Birmingham
Birmingham, AL 35294

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14. ABSTRACT

Bone is a common site of metastasis in breast cancer patients, leading to serious clinical consequences and a poor prognosis. The molecular mechanisms regulating this preferential metastasis of breast cancer to bone have not yet been fully elucidated. Our lab has found that CD68 is highly expressed in certain breast cancer cell lines and demonstrated that expression is correlative with bone adhesion ability. We hypothesized that CD68 mediates attachment of breast cancer cells onto bone matrix, subsequently regulating bone metastasis. Using stable shRNA (short hairpin RNA) to knock down CD68 expression in breast cancer cells and forcing expression of CD68 in breast cancer cell lines that do not endogenously express it, it is suggested that CD68 is not a critical adhesion molecule. However, because CD68 is a lysosomal protein, it may be involved in cell viability, particularly through autophagy. The ongoing studies may further provide more direct and convincing data.

15. SUBJECT TERMS
Breast Cancer Bone Metastasis, CD68, Bone Adhesion

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Introduction

Bone is a common site of metastasis in breast cancer patients, leading to serious clinical consequences and a poor prognosis. The molecular mechanisms regulating this preferential metastasis of breast cancer to bone have not yet been fully elucidated. Using a Phage Display-based approach by biopanning with bone slices, our lab identified macrosialin as a potential mediator of osteoclast adhesion to bone. Interestingly, we also found that CD68, the human homologue of macrosialin, is highly expressed in certain breast cancer cell lines. We hypothesized that CD68 mediates attachment of breast cancer cells onto bone matrix, subsequently regulating bone metastasis. In my proposal, I demonstrated that expression of CD68 is significantly increased in breast cancer cell lines that exhibit bone metastasis capacity, as compared to breast cancer cell lines that do not metastasize to bone. Preliminary data suggested that knocking down expression of CD68 in breast cancer cells decreased adhesion to bone. The purpose of this research is to further characterizing this interaction by using stable shRNA (short hairpin RNA) to knock down CD68 expression in breast cancer cells and determining bone metastatic capability *in vitro*, as well as *in vivo*. Additionally I proposed to force expression of CD68 in breast cancer cell lines that do not endogenously express it, in order to determine if CD68 will render them capable of attachment to bone. These ongoing studies may further provide direct and convincing data. As discussed with my contract specialist, Jesse Hoffman, I also took my maternity leave from September to mid-November.

Body

Specific Aim 1: Further characterize the role of CD68 in breast cancer cell attachment on bone in vitro

a. Develop stable long-term down-regulation of CD68 by siRNA in breast cancer cell lines MDA-MB-231 and MDA-MB-435. Examine if siRNA down-regulation of CD68 leads to reduced capacity to attach onto bone (Months 1-12)

I first addressed this issue by comparing the cell adhesion abilities of breast cancer lines with different intrinsic levels of CD68 and found that CD68 levels correlate with attachment of breast cancer cells onto bone *in vitro*. Breast cancer cells lines MCF-7, MDA-MB-231, MDA-MB-435, and MDA-MB-468 were cultured to 90% confluency and then lifted using 2mM EDTA. In order to determine the optimal parameters of the bone adhesion assay, four different timepoints were chosen and cells were cultured under three different media conditions (DMEM only, DMEM with complete levels of serum, and PBS). After incubation, cells were lysed for luciferase activity to determine the number of cells able to attach to the bone (**Figure 1**). Experiments had an n-value of 3, and were repeated two times.

To further explore CD68's role in cell adhesion to bone, I forced expression of the two human CD68 isoforms, CD68.1 and CD68.2, in MCF-7 and MDA-MB-468, the breast cancer cell lines with very low endogenous expression of CD68. The functional differences between the two isoforms are currently unknown. Full-length human CD68 cDNAs were cloned into the pMX-puro vector and transiently transfected into 293GPG cells to produce virus. MCF-7 and MDA-MB-468 were then infected with the CD68 virus and selected for stable expression using puromycin. **Figure 2a** shows a Western Blot confirming CD68 expression at the protein level of both isoforms in both breast cancer cell lines. Cell adhesion to bone assays were performed and found that nothing more than background levels of luciferase activity occurred (as shown in **Figure 2b and c**), suggesting that forced expression of CD68 was not sufficient to allow MCF-7 and MDA-MB-468 bone adhesion.

In the original proposal, I showed that CD68 antibody blocked interaction between bone and MDA-MB-231 breast cancer cell lines, but not MDA-MB-435. I proposed that MDA-MB-435s were not affected because, unlike MDA-MB-231, CD68 expression on the cell surface of MDA-MB-435s was not detectable. In order to look more specifically at this interaction, successful stable knockdown of CD68 in breast cancer cell lines MDA-MB-435 and MDA-MB-231 was achieved using shRNA. Corresponding control cells were also made using scrambled shRNA. These cells were then incubated on bovine bone slices for 1 hour, as determined by the timepoint experiment in figure 1, and then lysed for luciferase activity to determine the number of cells able to attach by preparing a standard curve. However, deviations within the experiment were a cause for concern. I attempted to address this problem in many different ways. In addition to trying different cell numbers, I also tried different ways of preparing the bone slices. Initially our lab attempted to use the same area of each bone chip to culture the cells by drawing a circle by tracing with a wax pencil to contain the media. Later, I attempted to prepare bone slices of identical sizes by using a lathe to file a piece of bone into a cylinder and cutting slices from the cylindrical bone. I also attempted to use a hole-puncher in order to obtain slices that were the same size, but found that often times this would just fracture the bone. Finally, I cut slices of cortical bone that were of similar size and then used Adobe photoshop to calculate the exact area of each bone slice. I could then normalize the data by finding the number of cells per squared millimeter of bone. This method eliminated the need to create bone slices that were identical in size, which was functionally impossible due to the composition of the bone. Additionally, the assays were initially performed by lysing the cells on the bone and then moving aliquots of the lysate to a new plate in order to determine the luciferase activity. However, the results were not consistent using this method, regardless of cell number plated or length of incubation. To circumvent this issue, I collaborated with Dr. Kurt Zinn's lab in order to use their luminometer. This provided me the ability to lyse the cells directly on the bone slices and be able to determine luciferase activity without having to aliquot the lysate. However, these assays revealed that the stable knockdown of CD68 did not affect the ability of the cells lines to adhere to the bone (**Figure 3).** I then repeated the CD68- antibody adhesion studies to determine whether the initial results were affected by the adhesion assays themselves. I found that any antibody treatment might be confounding when used in bone adhesion assay (**Figure 4**). Therefore, more specific techniques such as the stable shRNA experiments will yield more reliable data.

b. Quantify and compare CD68 expression levels in normal mammary epithelial cells, breast cancer tissues from primary sites and breast cancer tissues from bone metastasis (Months 2-24).

I have applied for and obtained the Institutional review board (IRB) approval to proceed with this portion of the research. I am currently awaiting the normal and diseased tissues from our official collaborator Dr. Hue Luu in the Department of Surgery at the University of Chicago. Upon obtaining the tissues, CD68 expression will be examined by Immunohistochemistry.

c. Identify specific regions in the CD68 extracellular domain that are required for binding to bone to facilitate the elucidation of the molecular basis of the breast cancer -bone interaction and, more importantly, help develop more potent and specific monoclonal blocking antibodies that may be used as therapeutics for preventing and treating breast cancer bone metastasis (Months 8-18).

Plans of developing deletion mutants in order to determine the specific regions of bone interaction have been suspended due to the lack of association I found in Specific Aim 1 part A between CD68 and bone adhesion.

Task 2: Investigate whether CD68 is involved in breast cancer bone metastasis in vivo using animal models (Specific Aim 2) Months 12-34.

UAB's Institutional Animal Care and Use Committee (IACUC) have approved the proposed animal studies for the second and third year of this research in the second Specific Aim. These studies will begin when there is confirmation of CD68's role using *in vitro* cancer assays.

Additional research to redirect aims associated with the project:

Due to the lack of association I discovered between expression of CD68 and bone adhesion, I further investigated potential roles of CD68 that would explain the correlation between high CD68 levels and cancer recurrence and/or bone metastatic ability. It is also important to recall that CD68 has already been shown to be of clinical importance in breast cancer as it is among the 16 different genes on the Oncotype DX profile to predict risk of breast cancer recurrence (1). Increased expression of CD68, although the function remains unknown, in breast cancer tumors is correlated with an increased rate of recurrence, with CD68 having a hazard ratio of 1.11, which is the sixth highest estimate of relative risk among the 16 genes analyzed in Oncotype DX (1;2). This further supports my hypothesis that CD68 plays a role in the metastasis of breast cancer to bone.

For breast cancer bone metastasis to occur, it is essential that breast cancer cells have enhanced and/or long-term viability in the bone microenvironment. Autophagy has been proposed to be a pro-survival factor in cancer during starvation periods and/or hypoxic periods in order to delay apoptosis or non-apoptotic programmed cell death, such as autophagic cell death or lysosomal cell death. Autophagy is a "self-eating" process a cell can undergo in order to enhance viability during nutrient deprivation and is especially activated during amino acid starvation (3). Some cancer cells can undergo autophagy until they are just 1/3 of their original size but can resume proliferation within 24 hours if nutrients are restored (4). The autophagic pathway is a highly conserved pathway mediated by a double-membraned sac termed the autophagosome, an organelle formed by a small vesicular sac enclosing portions of the cell's cytoplasm. The autophagosome fuses with a lysosome to form an autolysosome, which degrades the materials within to be recycled in the cytoplasm or used for energy production (5). Therefore, lysosomes are an essential component of the autophagic process and could be implicated in long-term cell viability. Importantly, LAMP1 and LAMP 2, of which CD68 belongs to the same family, are involved in autophagy by regulating fusion of the lysosome to the autophagosome. LAMP regulation of autophagy is accomplished in at least three ways: redistributing the lysosomes within the cell, altering motility by cholesterol accumulation, and modifying Rab7 association with phagosomes, which is a protein necessary for phagolysosome fusion (6;7). Overexpression of LAMPs has been shown to protect cells from autophagic cell death, whereas decreased levels of LAMP1 and LAMP2 increase susceptibility to lysosomal cell death (8,9). Another LAMP family member, LAPTM4B, was recently shown to play a pro-survival role in breast cancer by increasing resistance to anthracycline chemotherapies, by reducing drug entry into the nucleus (10). Therefore, members of the LAMP family may be important in supporting metastasis by enhancing long-term survival in breast cancer. Additionally, highly malignant tumor cells are found to have an increased cell surface expression of LAMP family members (9). I propose that because CD68 is a LAMP family member, it is involved in regulating autophagy to enhance viability in nutrient deprived conditions and/or lysosomal trafficking of drugs within the cell. First, I will address this by characterizing CD68's role in cell viability by examining cells under a number of cell stressors while controlling expression of CD68 by siRNA or forced expression. Next, I will determine if CD68's effects are regulating apoptotic and/or autophagic cell death pathways. Importantly, I will further investigate CD68's potential role in regulating autophagy, as it is a lysosomal associated process that has been previously linked to cancer cell survival. Lastly, I will determine if CD68 acts as a chemoresistant factor.

Key research accomplishments

- CD68 expression levels in MDA-MB-231, MDA-MB-435, MDA-MB-468, and MCF-7 breast cancer cell lines positively correlate with cells' ability to adhere to bone slices
- Stable knockdown of CD68 was achieved using shRNA
- Adhesion to bone was not affected when expression of CD68 was inhibited using shRNA in either MDA-MB-231 or MDA-MB-435.
- Forced expression of high levels of CD68 was achieved in MDA-MB-468 and MCF-7, lines that intrinsically express very low levels of CD68.
- Different techniques of bone adhesion assays were performed to overcome experimental inconsistencies

Reportable outcomes

Publications related to aims:

- None

Publications not related to aims:

- Ashley JW**, McCoy EM**, Clements DA, Shi Z, Chen T, Feng X. Development of cell-based high-throughput assays for the identification of inhibitors of receptor activator of nuclear factor-kappa B signaling. *Assay Drug Dev Technol* 9(1):40-49, 2011.

Abstracts selected for poster presentations

- "Potential Role of CD68 in breast cancer bone metastasis" **Erin M McCoy**. Zhenqi Shi, Xu Feng. *American Society for Bone and Mineral Research*. Poster presented in Toronto, Canada in the cancer section; October 15-19, 2010.

Awards:

- Pathology graduate student travel grant awarded for the presentation at American Society for Bone and Mineral Research; October 15-19, 2010- $1000.
- First place - poster presentations for Pathology Graduate Student Research Day, October 2010. "Potential Role of CD68 in breast cancer bone metastasis." Erin M. McCoy. *Dept of Pathology, University of Alabama at Birmingham.*

Other:

- Attended Annual meeting of American Association for Cancer Research, Florida, USA; April 2011.
- Successful passed my doctoral candidacy examination by my thesis committee.
- Took maternity leave September 1 – Nov 17th, 2011.

Conclusions

Through the course of this research so far, I have been able to determine that CD68 is upregulated in certain breast cancer cells lines (MDA-MB-231 and MDA-MB-435) that have a higher capacity adhere to bone slices *in vitro* and to metastasize to bone *in vivo*. Paired with the clinical use of CD68 as a recurrence risk factor for breast cancer, the need to find the specific role(s) for CD68 is critical in order to better understand and treat breast cancer metastasis. Although initially we found CD68 through a phage display bone adhesion assay and there is a correlation between expression of CD68 and bone adhesion abilities of breast cancer cells lines, CD68 does not appear to be the critical adhesion molecule that allows breast cancer to attach to bone. Therefore, I began a thorough literature review to investigate other

potential roles that CD68 may play in breast cancer metastasis and determined that, because CD68 is a LAMP, there is great potential for it to be involved in the pro-metastatic autophagy and/or chemotherapeutic resistance through autophagy.

First, I will address this by characterizing CD68's role in cell viability by examining cells under a number of cell stressors while controlling expression of CD68 by siRNA or forced expression. Next, I will determine if CD68's effects are regulating apoptotic and/or autophagic cell death pathways. Importantly, I will further investigate CD68's potential role in regulating autophagy, as it is a lysosomal associated process that has been previously linked to cancer cell survival. Lastly, I will determine if CD68 acts as a chemoresistant agent.

Cell Viability: I will use the MDA-MB-231 and MDA-MB-435 breast cancer cell lines in which I have achieved stable knockdown of CD68 (**Figure 3a**) to determine viability levels using stressors such as serum starvation. I will also use the MCF-7 and MDA-MB-468 breast cancer cell lines, which express minimal endogenous CD68 and compare cell viability to the MCF-7 and MDA-MB-468 with forced expression of CD68 (**Figure 2a**). I expect the knockdown of CD68 to render MDA-MB-231 and MDA-MB-435 less resistant to serum starvation and forced expression of CD68 to increase viability in MCF-7 and MDA-MB-468 when subjected to nutrient deprivation. However, failure of the forced expression model would not necessarily discount CD68 as a viability factor because trafficking within the model may be altered.

Apoptosis/Autophagy: I will further address the role of CD68 in breast cancer cell viability in order to distinguish between apoptotic pathways and autophagic pathways. To this end, I will use the data from the cell viability aim to determine at which time points and serum concentrations cell death is occurring and will then lyse cells in these conditions for Western Blotting of Akt/protein kinase B, Caspase-3, and cleaved Poly (ADP-ribose) polymerase (PARP) to determine if apoptosis is involved. Importantly, the MCF-7 breast cancer cell line has truncated Caspase-3 not detectible by Western Blotting, so instead Caspase-7 will be used.

Autophagic pathways will be determined by LC3 immunoblotting, GFP-LC3 puncta formation and quantification of acidic vesicular organelles with acridine orange staining. Morphologic changes by serum starvation, including cytoplasmic and nuclear shrinkage, chromatin condensation, cytoplasmic blebbing with maintenance of the integrity of cell membrane, will also be used to determine if autophagy is present. Cells will be microscopically examined for accumulation of autophagic compartments, which has been shown in other cases of LAMP family members disruption (11). Autophagy will be induced in the parental cells and the CD68-low (by CD68 siRNA) cell lines by rapamycin to determine if inhibition of CD68 decreases the ability of the cells to induce autophagy (12). All antibodies needed are commercially available. Because CD68 is a LAMP family member, I expect autophagy to be involved in the increased viability during nutrient deprivation. Whether autophagic, necrotic, or apoptotic cell death pathways are occurring is yet to be determined but all could be delayed by autophagy.

Chemoresistance: To determine if CD68 acts as a chemoresistant agent, chemotherapeutic drugs (Doxorubicin, Paclitaxel, and Cisplatin) will be added after 24 hours after seeding the above cell lines. Cells will be exposed to a series of concentrations of individual drugs for an additional 48 hours. The percentage of viable cells in drug-treated wells as compared to media-treated control wells will be platted as a drug-dose dependent survival curve. The IC_{50} (dose causing 50% reduction of viable cells) will be determined for each cell line. I expect that increased CD68, whether endogenous or forced, may increase resistance to some chemotherapeutic agents.

In summary, I expect these additional studies to provide insights into CD68's role as a mediator of cell viability by exploring its regulation of apoptosis and/or autophagy. Because it is a LAMP family

member, there is a good possibility that CD68 plays a role in the lysosomal associated process autophagy. However, the exact role of autophagy in breast cancer cells is currently unclear. Through this aim, I will determine if autophagy leads to enhanced viability during nutrient deprivation or if the process leads to autophagic cell death. Likely, the role of autophagy may be involved by enhancing viability during nutrient deprivation until it exhausts its own resources and succumbs to autophagic or apoptotic cell death. While it is unlikely CD68 plays a direct role in proliferation, if proliferation is indeed implicated, I will determine which stage of the cell cycle CD68 is activating or inhibiting and then investigate the specific modulations of the cell cycle. Alternatively, it is possible that both viability and proliferation are affected by CD68, especially because proliferation may be regulated by the induction of autophagy.

Our lab is highly proficient in molecular cloning, including siRNA knockdown technology, viral constructs, and forced expression of genes. Additionally, I have personal experience with these techniques as well as Immunohistochemistry, Western blotting, Flow cytometry analyses, and microscopic examinations for morphological changes of autophagy.

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Appendices

Accepted abstract for American Society for Bone and Mineral Research

"Potential Role of CD68 in breast cancer bone metastasis" Erin M McCoy. Zhenqi Shi, Xu Feng. *American Society for Bone and Mineral Research*. Poster presented in Toronto, Canada in the cancer section; October 15-19, 2010.

Bone is a common site of metastasis in breast cancer patients, leading to serious clinical consequences and a poor prognosis. The molecular mechanisms regulating this preferential metastasis of breast cancer to bone have not yet been fully elucidated. Using a Phage Display-based approach by biopanning with bone slices, our lab recently identified macrosialin as a key mediator of osteoclast attachment to bone. Interestingly, we also found that CD68, the human homologue of macrosialin, is highly expressed in certain breast cancer cell lines. We hypothesize that CD68 mediates attachment of breast cancer cells onto bone matrix, subsequently regulating bone metastasis. Here we demonstrate that expression of CD68 is significantly increased in breast cancer cell lines that exhibit bone metastasis capacity, as compared to breast cancer cell lines that do not metastasize to bone. Furthermore, anti-CD68 antibody significantly blocks the attachment of breast cancer cells to bone *in vitro*, supporting that CD68 plays a key role in breast cancer cell interaction with bone. These data indicate that CD68 plays a critical role in mediating breast cancer cell attachment to bone. We are currently characterizing this interaction by using shRNA to knock down CD68 expression in breast cancer cells and determining bone metastatic capability *in vitro*, as well as *in vivo*. Additionally we are also expressing CD68 in breast cancer cell lines that do not endogenously express it, in order to determine if CD68 will render them capable of attachment to bone. These ongoing studies may further provide direct and convincing data.

CURRICULUM VITAE

Erin Mills McCoy, B.S.

DEMOGRAPHIC INFORMATION

Current Position

Howard Hughes Med-to-Grad Pre-doctoral fellow – Department of Pathology, Division of Cellular and Molecular Pathology, University of Alabama at Birmingham

Education:

6/07 - present	Howard Hughes Med-to-Grad Pre-doctoral fellow Department of Pathology Division of Cellular and Molecular Pathology University of Alabama at Birmingham Overall GPA – 4.0
5/03 – 5/07	B.S. - Biological Sciences B.S. – Microbiology Minor – Biochemistry Mississippi State University; Starkville, MS Overall GPA: 3.97, 143 hours completed
08/06	GRE: Verbal: 530; Quantitative: 740; Total 1270

Honors and Activities:

Graduate:

Department of Defense Pre-doctoral Breast Cancer Fellowship award: 2011-2014. $124,950.

Pathology graduate student travel grant awarded for the presentation at American Society for Bone and Mineral Research; October 15-19, 2010- $1000.

Carmichael Scholarship for Academic Excellence: 2009-2011; competitively renewed for 2010-2011: Awarded $22,000/year

Most Outstanding Graduate Student through Service Award: 2008-2009 as chosen by a vote from all peers in the Pathology Graduate Student Department

Second place - oral presentations for Pathology Graduate Student Research Day, October 2008. "Characterizing the role of CD68 in mediation of breast cancer attachment to bone." Erin M. McCoy. *Dept of Pathology, University of Alabama at Birmingham.*

Howard Hughes Med-to-Grad Fellowship 2007-present: Ph.D. Training fellowship for Translational Research and Drug Discovery at University of Alabama at Birmingham. This fellowship is awarded to the top incoming students in the biomedical sciences graduate programs and supports the students for the first 16-months of their graduate career.

Undergraduate:

President's list (every semester) - Mississippi State University
Phi Beta Kappa, Society of Scholars, inducted 2006
Phi Kappa Phi, inducted 2006
Mortar Board, Treasurer 2006 - 2007
Mabry-Clark Memorial Scholar, selected as one of two students from MSU, 2006
Sigma Phi Lambda service sorority, Vice President 2006
Fred and Mary Koch Scholarship, 2004-present
National Merit Georgia Pacific Scholarship, 2003-2007
Robert C. Byrd Scholarship, 2003-2007
Mississippi Eminent Scholars Grant, 2003-2007
MSU Academic Scholarship, 2003-2007

RESEARCH ACTIVITIES

Major Research Interests:

July 2008 – present. We are interested in understanding the molecular and cellular mechanisms of breast cancer metastasis to bone; specifically adhesion molecules that allow interactions between breast cancer cells and bone surface and viability factors allowing for survival in the bone microenvironment.

Past Research Experience:

Research Assistant: Mississippi Genomic Exploration Laboratory, Department of Plant and Soil Sciences, Mississippi State University, August 2006 – March 2007: Participated in research to complete a bacterial artificial chromosome library for the genomic sequencing of the loblolly pine tree species.

Research Assistant: Department of Biological Sciences, Mississippi State University, August 2006 – Jan 2007: Participated in FIV research to monitor acute serological and virological changes in the peripheral circulation of FIV-infected cats.

Program for Research Experience in Pathology, University of Alabama at Birmingham, May 2006 – July 2006: Successfully constructed a luciferase reporter to be further used in a cell-based high throughput screening for anti-resorptive compounds.

Research Assistant: Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, May 2005 – May 2006: Participated in research on the risk factors of *Salmonella* in poultry farms and processing plants.

EDUCATIONAL ACTIVITIES

Classroom Instruction:

English as a Second Language Teacher Training: 14 hour teacher training class, received certification January 30, 2010. To date: 12 hours in classroom.

Instructor: General Chemisty, CH121-03, Birmingham Southern College Department of Chemistry, Fall 2008.

Teaching Assistant: Practices in the Physiology of Reproduction, PHY 4611, Mississippi State University-College of Veterinary Medicine, Fall 2005.

Accepted Abstracts/Presentations:

"Potential Role of CD68 in breast cancer bone metastasis" **Erin M McCoy**. Zhenqi Shi, Xu Feng. *American Society for Bone and Mineral Research*. Poster presented in Toronto, Canada in the cancer section; October 15-19, 2010.

"Potential Role of CD68 in breast cancer bone metastasis." **Erin M McCoy**. Zhenqi Shi, Xu Feng. Poster presented for Pathology Graduate Student Research Day, October 2010. *Dept of Pathology, University of Alabama at Birmingham.*

"Regulation of Breast Cancer Attachment to Bone by CD68" **Erin M. McCoy,** Zhenqi Shi, Xu Feng. *Department of Pathology, University of Alabama at Birmingham.* Poster presented at UAB Comprehensive Cancer Retreat, Birmingham, AL; November 2008.

"Characterizing the role of CD68 in mediation of breast cancer attachment to bone" **Erin M. McCoy**, Zhenqi Shi, Xu Feng. Oral presentation (2nd place) – Pathology Graduate Student Research Day, October 2008.

"Cell-Based Assays for Identifying Inhibitors of Bone Loss" JW Ashley, Z Shi, **EM McCoy**, DA Clements, X Feng. *Department of Pathology, University of Alabama at Birmingham.* Poster presented at the Regional Peer Cluster Conference for Howard Hughes Med-to-Grad Fellows, Birmingham, AL; August 2008.

"Disruption of VPS34 expression *in vivo* reveals its novel role in embryonic development." **Erin M. McCoy** and Jianhua Zhang. *Department of Pathology, University of Alabama at Birmingham.* Oral presentation open to all departments, May 2008.

"Deletions of alpha-Calmodulin Kinase II in Osteoblasts Reveals Its Novel Role in Bone Remodeling" **Erin M. McCoy** and Majd Zayzafoon. *Department of Pathology, University of Alabama at Birmingham.* Oral presentation open to all departments, March 2008.

"Identification of c-Src Motifs Essential for Osteoclast Function." **Erin M. McCoy** and Xu Feng. *Department of Pathology, University of Alabama at Birmingham.* Oral presentation open to all departments, December 2007.

"Analysis of Recombinant ssM13mp18 RF pGFPuv Using DNA sequencing and Detection of pGFPuv and pUC by Southern Blotting. **Erin E. Mills.** Oral presentation – Department of Biochemistry, Mississippi State University, April 2007.

"Kinetic Studies of Chicken Muscle Lactate Dehydrogenase: Inhibition using Oxalate, Irreversible Inhibition using Butanedione, and pH Characterization. **Erin E. Mills.** Oral presentation – Department of Biochemistry, Mississippi State University, Dec 2006.

"Virological and Serological Consequences of Acute Feline immunodeficiency Virus (FIV) Infection" Crystal Boudreaux, **Erin Mills,** Matt Bramuchi, Veronica Scott, Nikki Lockett, Brittany Clay, and Karen S. Coats. *Department of Biological Sciences, Mississippi State University.* Poster presented at the South Central Branch of the American Society of Microbiology, Baton Rouge, LA; November 3-4, 2006.

"Construction of a Luciferase Reporter for Establishing a Cell-based High Throughput Screening Assay System to Identify New Antiresorptive Drugs" **Erin E. Mills**, Zhenqi Shi, and Xu Feng. *Department of Pathology, University of Alabama at Birmingham.* Poster presented at the closing ceremony for the *Program for Research Experience in Pathology, UAB,* July 2006.

Tutoring Positions:

Summer 2010

Summer peer mentor, McNair Scholars Research Program, UAB, Birmingham, AL

Fall 2009 – Spring 2011

Fundamentals of Biochemistry; McNair Scholars Program; UAB, Birmingham, AL

Fall 2006 – Summer 2007

Plant and Animal Biology. Mississippi State University; Starkville, MS

Summer 2004

Chemistry I & II, Organic Chemistry I. Jones County Junior College; Ellisville, MS

Judging Activities:

UAB Center for Community Outreach Development (CORD) Summer Science Institute Closing Ceremonies Poster Competition, Birmingham, AL; August, 2009.

ORGANIZATION ACTIVITIES

Institutional Committees:

August 2008 – Fall 2009

Senator representative for Pathology in the Graduate Student Association; chair of Career Day committee, member of Student Health committee.

July 2006 – May 2007.

Member of the Institutional Animal Care and Use Committee – Student body representative for Mississippi State University.

Professional Societies:

American Association for Cancer Research, 2009-present
Metastasis Research Society, 2008-present
American Association for the Advancement of Science, 2007 - present

Committees/Offices held

Team Captain for the UAB Pathology Department – Komen Breast Cancer Race for the Cure, October 2008 and October 2009, Birmingham, AL.

Co-chair and organizer for the First Annual Howard Hughes Medical to Graduate Fellow Peer Cluster Conference (UAB, Baylor, and Rice University), August 2008, Birmingham, AL .

Co-chair of Activities Committee for Howard Hughes Med-to-Grad Fellows. Activities include certification and opportunities for middle school and high school tutoring/mentoring programs to facilitate scientific interest at an early age.

Chair of Volunteer committee: Sigma Phi Lambda 2005-2007. Organized and assisted with events such as food and clothing drives, Hurricane Katrina outreach programs, and Habitat for Humanity programs.

OTHER EXPERIENCE:

Research Assistant: Atlanta Zoo, Atlanta, GA. December 2005. Assisted with a digestibility trial in pandas. Collected fecal matter and ortfs (rejected bamboo), sub-sampled and separated by plant species. Selected as one of two students from a pool of 135 students.

Dairy Farm Research Assistant: Dairy Research Center, Mississippi State University. May 2005 through December 2006. Assisted in the collection of milk samples for research and maintained the separation between different experimental groups within the herd.

Veterinary/Surgical Assistant: Southern Pines Animal Shelter, Hattiesburg, MS. May 2001 through December 2004: Collected ear, skin, and tissue cultures, performed stains, autoclaved surgical equipment. Gained familiarity with sterile and aseptic techniques.

SUPPORTING DATA

FIGURE 1

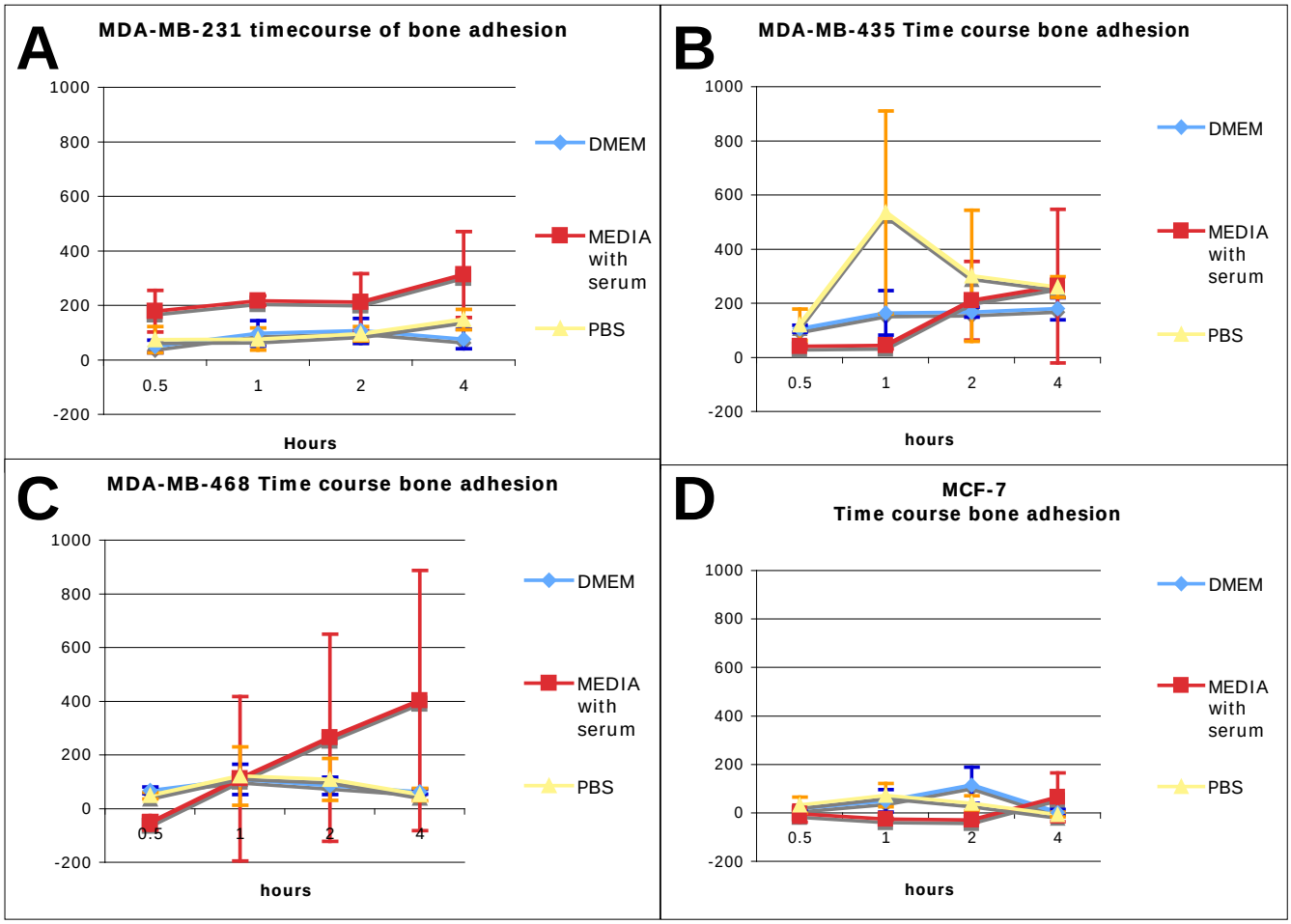

Figure 1. Bone adhesion assays to determine optimal times and culture conditions. Each cell line was cultured with the bovine cortical bone slices for four timepoints: 30 minutes, 1 hour, 2 hours, and 4 hours. At each timepoint, each cell line was tested at DMEM only, Media with serum, or PBS. Cells were then lysed and attached number of cells was calculated using luciferase activity. A) MDA-MB-231, B) MDA-MB-435, C) MDA-MB-468, and D) MCF-7.

FIGURE 2

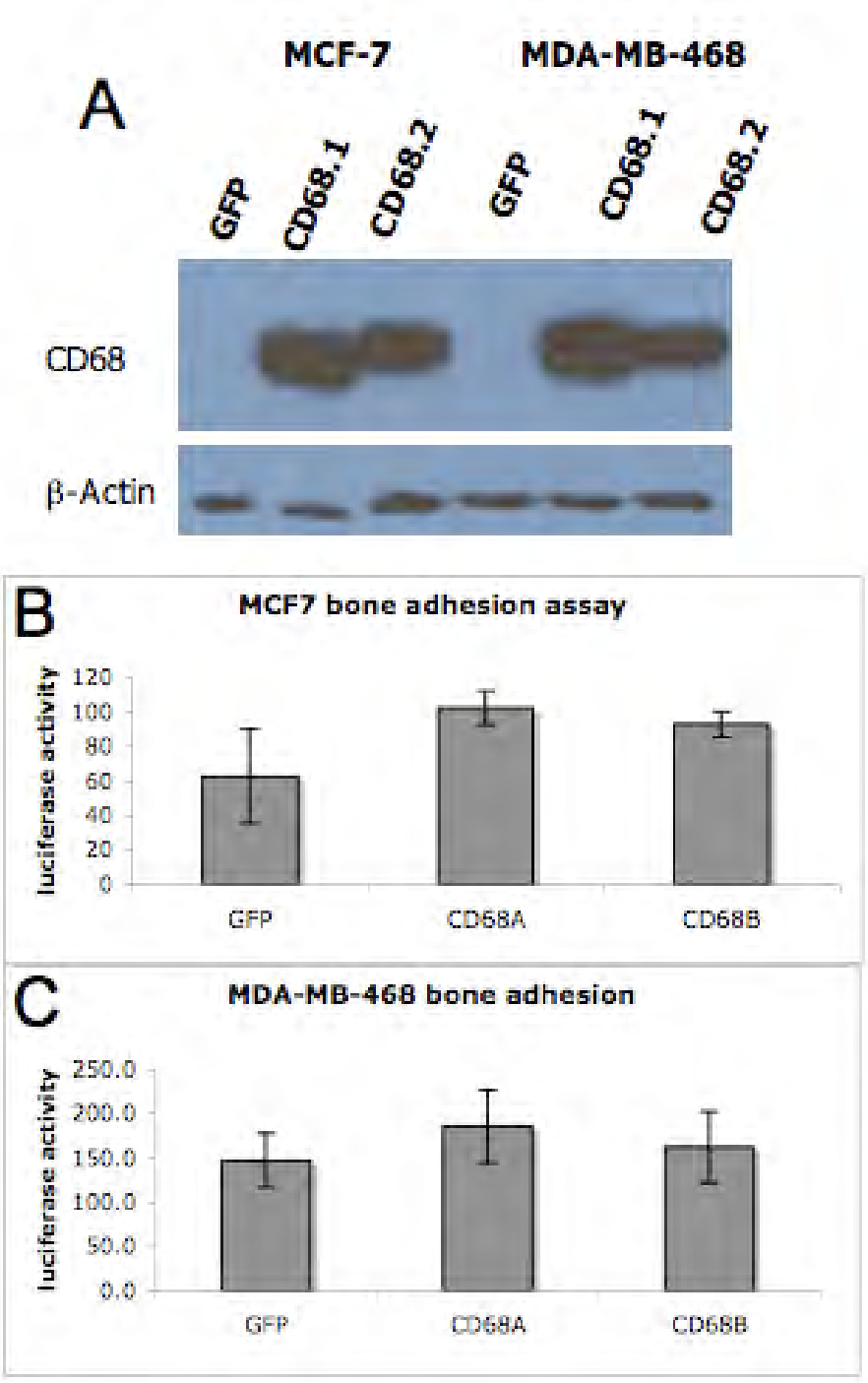

Figure 2. Bone adhesion assay using MCF-7 and MDA-MB-468 breast cancer cell lines with forced expression of CD68. A) Western blotting confirming forced expression of two isoforms of CD68 (68.1 and 68.2) in MCF-7 and MDA-MB-468. B) Bone adhesion assay of MCF-7, and C) MDA-MB-468, both showing background levels of luciferase.

Figure 3

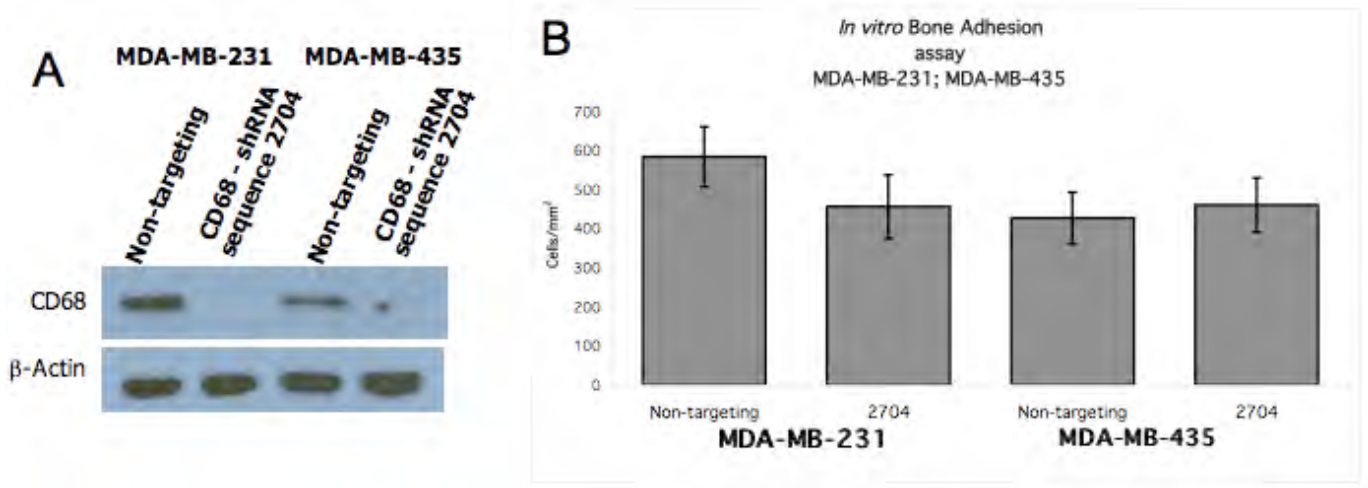

Figure 3. Bone adhesion assay using stable knockdown of CD68. A) Western blotting of CD68 protein (Santa Cruz antibody sc-17832, mouse monocloncal human CD68 (E-11) showing downregulation of CD68 by knockdown B) Bone adhesion assay comparing non-targeting to CD68-knockdown in MDA-MB-231 and MDA-MB-435. Error bars represent standard error, p-values were not significant (0.3 for MDA-MB-231 and 0.8 for MDA-MB-435)

Figure 4

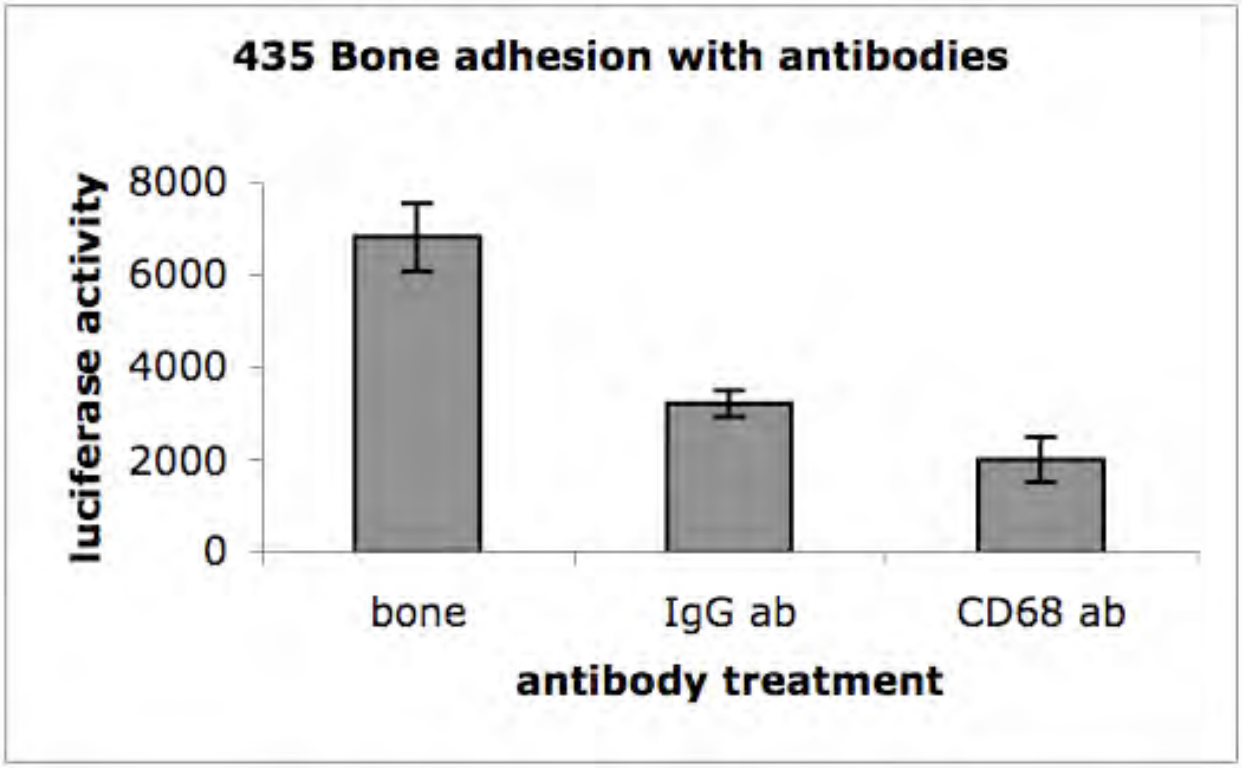

Figure 4: Bone adhesion assay showing decrease in adhesion with both the IgG control antibody as well as the CD68 antibody (Santa Cruz antibody sc-17832, mouse monoclonal human CD68)(E-11). Cells were treated for 30 minutes with 10μg of either antibody and then cultured on the bone slices for 1 hour. A decrease in adhesion was shown with the CD68 antibody, but confounded by the decrease in the IgG control antibody group.